J. Clin. Chem. Clin. Biochem.
Vol. 23, 1985, pp. 725–732

Determination of Theophylline in Saliva, Using Fluorescence Polarization Immunoassay (FPIA)

By *A. Niemann, M. Oellerich, G. Schumann* and *G. W. Sybrecht*

Institut für Klinische Chemie und Zentrum Innere Medizin, Medizinische Hochschule Hannover

(Received April 9, 1985)

Herrn Professor Dr. A. Delbrück zum 60. Geburtstag gewidmet

Summary: Theophylline concentrations were determined by fluorescence polarization immunoassay (FPIA) in saliva, serum and serum water of healthy volunteers and outpatients after administration of single theophylline doses, and after the administration of several doses in order to establish a steady state. The FPIA allowed rapid and reliable theophylline determinations in saliva and serum water (between-days coefficients of variation: < 3%; recovery: 95–103%). Salivary theophylline concentrations measured by FPIA in 30 samples agreed well with those determined by HPLC. Furthermore the results obtained by ultrafiltration for the concentration of unbound theophylline in serum water were in good agreement with those determined by ultracentrifugation. The binding of theophylline by serum protein rose by about 25%, when the pH of the samples was increased from 7.0 to 8.0. After adjusting the pH to 7.4, average values for theophylline binding to proteins at 25 °C ranged from 48.5 to 52.2% in serum samples from outpatients and healthy adults. Salivary theophylline concentrations correlated well with total and free serum theophylline concentrations in healthy adults and outpatients ($r = 0.90–0.98$). The theophylline concentration in saliva was on average about 20–30% higher than the unbound theophylline concentration in serum water. The saliva/serum concentration ratio of theophylline showed some intersubject variation (0.68 ± 0.08; range: 0.50–0.85). Using the mean saliva/serum concentration ratio of the patient group, steady state serum theophylline concentrations were predicted from salivary levels with a mean error of 7.6% (range: 0.0–26.8%). The salivary theophylline concentration appears to be a suitable parameter for assessment of compliance, for identification of patients with inappropriate dosage, and for consequent dosage adjustment. Individual dosage optimisation, however, should be based on theophylline concentrations determined in serum.

Bestimmung von Theophyllin im Speichel mittels Fluoreszenz-Polarisations-Immunoassay (FPIA)

Zusammenfassung: Die Theophyllin-Konzentration in Speichel, Serum und Serum-Wasser von gesunden Probanden und ambulanten Patienten wurde nach Verabreichung von Theophyllin-Einzeldosen und im steady-state mittels Fluoreszenz-Polarisations-Immunoassay (FPIA) bestimmt. Der FPIA gestattete rasche und zuverlässige Bestimmungen von Theophyllin im Speichel und Serum-Wasser (Variationskoeffizienten von Tag zu Tag: < 3%; Wiederfindung: 95–103%). Die mit dem FPIA in 30 Proben gemessenen Theophyllin-Konzentrationen im Speichel stimmten gut mit den entsprechenden Resultaten der HPLC überein. Weiterhin ergab sich eine gute Übereinstimmung der mittels Ultrafiltration und Ultrazentrifugation bei der Bestimmung von ungebundenem Theophyllin im Serum-Wasser erhaltenen Ergebnisse. Bei einem Anstieg des pH der Probe von 7,0 auf 8,0 wurde eine Zunahme der Bindung von Theophyllin an Serumproteine um 25%

beobachtet. Nach Einstellung des pH auf 7,4 lagen die mit Serumproben von ambulanten Patienten und gesunden Erwachsenen gefundenen Mittelwerte für die Proteinbindung von Theophyllin bei 25 °C zwischen 48,5 und 52,2%. Die Theophyllin-Konzentration im Speichel korrelierte bei gesunden Erwachsenen und ambulanten Patienten gut mit der freien und der Gesamt-Theophyllin-Konzentration im Serum (r = 0,90−0,98). Die Theophyllin-Konzentration im Speichel lag im Mittel etwa 20−30% höher als die freie Theophyllin-Konzentration im Serum-Wasser. Das Speichel/Serum-Konzentrationsverhältnis von Theophyllin zeigte eine gewisse interindividuelle Variabilität (0,68 ± 0,08; Spannweite: 0,50−0,85). Unter Verwendung des durchschnittlichen Speichel/Serum-Quotienten der Patientengruppe ließen sich die steady-state-Theophyllin-Konzentrationen im Serum anhand der entsprechenden Konzentrationen im Speichel mit einem Fehler von 7,6% (Spannweite: 0,0−26,8%) vorhersagen. Die Theophyllin-Konzentration im Speichel erscheint als Kenngröße zur Abschätzung der Patienten-Compliance, zur Erkennung von Patienten mit inadäquater Dosierung und zur nachfolgenden Dosisadaptation geeignet. Die individuelle Optimierung der Dosierung sollte jedoch anhand der im Serum bestimmten Theophyllin-Konzentration vorgenommen werden.

Introduction

The monitoring of theophylline serum concentrations is well established (1). However, conflicting results have been reported in the literature regarding the suitability of salivary theophylline concentrations for monitoring theophylline therapy (2−7). A non-invasive method for monitoring theophylline levels could play an important role, particularly in the treatment of nocturnal asthma, where circadian variations of airway function have to be taken into account (8). Saliva specimens can be collected by the patient at any preset time during the interval between doses outside the clinic. This enables the physician to estimate the actual serum theophylline level from salivary theophylline concentrations at the time when asthmatic attacks or toxic symptoms occur.

In the present study we re-evaluated the question of whether the salivary theophylline concentration is a suitable parameter for monitoring theophylline therapy. We therefore compared salivary theophylline concentrations with free and total serum theophylline concentrations determined in samples from outpatients and healthy volunteers receiving the drug. In addition we investigated the suitability of fluorescence polarization immunoassay (FPIA) for rapid and reliable determinations of salivary and unbound serum theophylline concentrations.

Materials and Methods

Origin of specimens

Serum and saliva specimens (n = 80) were obtained from 10 healthy volunteers, after administration of a single dose of theophylline ethylenediamine solution (Euphyllin®, 5.6 mg/kg anhydrous theophylline) orally or intravenously over a period of 15 min. One week later the same volunteers received a single dose of choline theophyllinate tablets (Euspirax®, 6.0 ± 0.6 mg/kg anhydrous theophylline). Venous blood and mixed stimulated saliva was taken 0.5, 1, 2, 3, 5, 7, 9 and 12 h after drug administration.

Furthermore, serum and saliva specimens (n = 56) were obtained at steady state from 8 healthy volunteers who had received choline theophyllinate tablets (Euspirax®, 5.7 ± 0.7 mg/kg anhydrous theophylline) at 8.00 a. m. and 8.00 p. m. for three days. Blood and saliva specimens were taken 0, 2, 4, 6, 8, 10 and 12 h after the last administration of the tablets. Additional serum and saliva specimens (n = 30) were obtained from 30 outpatients at steady state. In addition to theophylline, some of these patients received β_2 sympathomimetics. In contrast to the volunteers the patients were not instructed to abstain from methylxanthine-containing beverages and chocolate.

Collection of saliva specimens

Mixed saliva specimens (2−3 ml) were obtained during each blood sampling in small polystyrol tubes (Sarstedt, Rommelsdorf, FRG). Salivation was stimulated by chewing a piece of Parafilm® (American Can Comp., Greenwich, U. S. A.) for about 3 minutes. There was no noticeable adsorption of theophylline to the polystyrol tubes and Parafilm® used. Salivary pH was measured using a digital pH meter. The mean pH value of all stimulated saliva specimens of the outpatients and volunteers was 6.6 ± 0.6 (range: 5.7−7.7). Saliva was centrifuged at 7000 g for 3 minutes to separate mucus and cell debris. The supernatant was used for the theophylline determination.

Separation of unbound theophylline

The serum samples (n = 246) were kept frozen for about one week. Before analysis the pH of the samples had to be adjusted to pH 7.4 with microliter quantities of 1.0 mol/l HCl, as the pH of the samples increased during storage to values between 7.7 and 8.0. For separation of unbound theophylline ultrafiltration devices (Centrifree Micropartition System, Amicon Corp., Danvers, U.S.A.) were filled with 1 ml of each serum sample and centrifuged at 2000 g for 10 minutes (temperature, 25 °C). The ultrafiltrates were collected and used for the determination of unbound theophylline. The absence of protein from each ultrafiltrate was checked by using a dip stick (Albustix, Ames, Elkhart, U. S. A.). In addition, unbound theophylline concentrations were determined in the 105 000 g supernatant of these serum samples (n = 32) obtained by ultracentrifugation as previously described (9).

Determination of binding of theophylline to serum protein at different pH values

The pH of human pool serum was varied from 7.0 to 8.0. The samples were adjusted to the respective pH with 1.0 mol/l HCl or NaOH. The dilution of the serum samples due to titration was less than 1%. Unbound theophylline was separated by ultrafiltration as described above.

Fluorescence polarization immunoassay (FPIA)

Theophylline determinations in serum, saliva and serum water were carried out by FPIA (Theophylline Reagent Pack, Abbott Laboratories, North Chicago, U. S. A.) using the TDx® Fluorescence Polarization Immunoassay System according to the instructions of the manufacturer.

High performance liquid chromatography (HPLC)

Theophylline concentrations in saliva specimens (n = 30) from volunteers and outpatients were measured by an HPLC method as described elsewhere (10, 11).

Statistical analysis

The statistical evaluation of the results was based on the standardised principal component analysis (12) and the significance of the bias $\bar{x} - \bar{y}$ (paired t-test). Unless stated otherwise mean values with standard deviation are given.

Results

Precision of FPIA

At theophylline concentrations in saliva between 7.9 and 10.9 mg/l and in serum water between 5.3 and 10.1 mg/l the between-days coefficients of variation of single determinations ranged from 1.9–2.5% (tab. 1).

Tab. 1. Between-days precision of FPIA for the determination of theophylline in saliva and ultrafiltrate.

Theophylline in saliva[a]			Theophylline in ultrafiltrate[b]		
$\bar{x}$ (mg/l)	CV (%)[c]	n[d]	$\bar{x}$ (mg/l)	CV %[c]	n[d]
7.9	1.9	14	5.3	2.1	10
10.9	2.5	14	10.1	2.2	10

[a] saliva samples from two outpatients receiving theophylline
[b] serum samples from two outpatients receiving theophylline
[c] mean value ($\bar{x}$) with coefficient of variation (CV)
[d] number of days

Tab. 2. The recovery of theophylline by FPIA in saliva and serum water.

Saliva				Serum water			
Theophylline weighed in (mg/l)	Found[1] (mg/l)	Recovery[1] (%)	n[2]	Theophylline weighed in (mg/l)	Found (mg/l)	Recovery (%)	n
10	9.7	97	4	10	9.8	98	4
6	6.2	103	4	6	6.2	103	4
4	4.1	102	4	4	3.8	97	4
2	1.8	95	4	2	1.9	95	4

[1] mean value
[2] number of contributing values

Accuracy of FPIA

Average recoveries of theophylline added to drug-free saliva and to the 105 000 g supernatant of drug-free human serum are shown in table 2. At theophylline concentrations in saliva or serum water between 2.0 and 10.0 mg/l the recovery ranged, with FPIA, from 95 to 103%.

Furthermore the results obtained by FPIA with 30 saliva samples from outpatients and volunteers agreed very well with those determined by HPLC (fig. 1). The mean value from FPIA was about 6% higher than that observed with HPLC. Although this difference was statistically significant ($p < 0.05$) it does not seem to be clinically relevant.

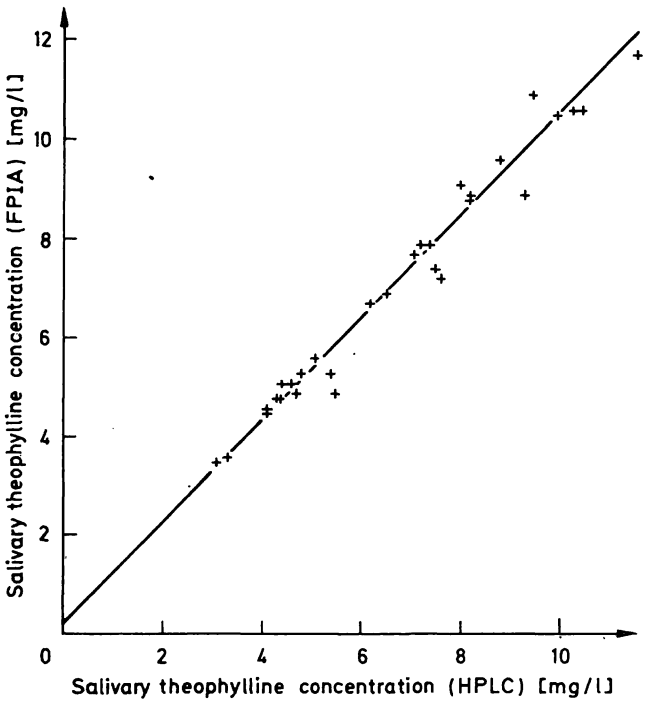

Fig. 1. Salivary theophylline concentrations as measured by HPLC and FPIA in samples from patients and volunteers (n = 30) receiving the drug.
[y = 1,02x + 0.26 mg/l; r = 0.98; $\bar{x}$ (s) = 6.7 (2.4) mg/l; $\bar{y}$ (s) = 7.1 (2.4) mg/l].

Comparison of ultrafiltration and ultracentrifugation for separation of unbound theophylline

A comparison was made of the results obtained by ultrafiltration and ultracentrifugation for the concentration of unbound theophylline in 32 serum samples from volunteers taking this drug. The results determined by both methods agreed very well (fig. 2). The mean values found were almost identical ($p > 0.05$).

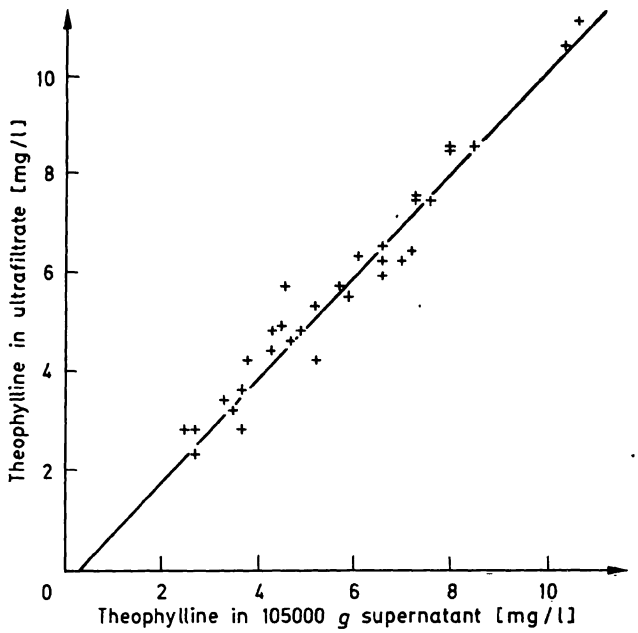

Fig. 2. Serum unbound theophylline concentration determined by FPIA in 105000 *g* supernatant and ultrafiltrate of samples (n = 32) from volunteers taking the drug. [y = 1.04x − 0.24 mg/l; r = 0.98; $\bar{x}$ (s) = 5.7 (2.1) mg/l; $\bar{y}$ = 5.7 (2.2) mg/l].

Effect of pH on binding of theophylline by serum protein

Binding of theophylline to serum proteins was found to be pH-dependent (fig. 3). The percentage of unbound theophylline decreased from about 60% at pH 7.0 to 35% at pH 8.0 in samples of human pool serum. The average values for the percentage of theophylline bound to serum proteins in samples from 10 healthy volunteers and 30 outpatients were 49.9 ± 3.1% and 48.5 ± 4.9%, respectively (temperature: 25 °C; pH: 7.4).

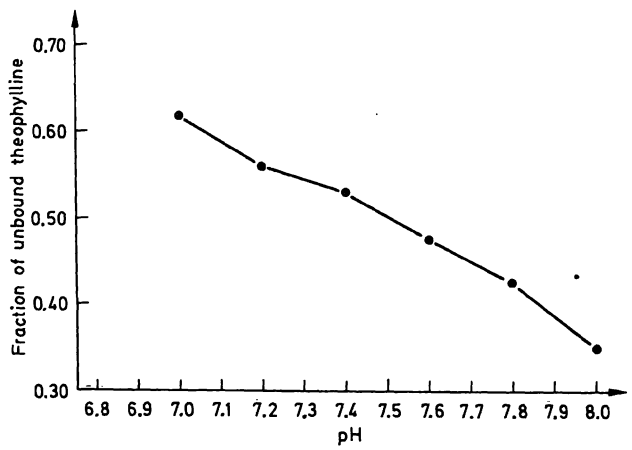

Fig. 3. Effect of pH on theophylline binding to serum proteins. Each point represents the average value of determinations on two samples of human pool serum.

Comparison of theophylline concentrations in saliva, serum and serum water

Averaged concentration-time curves of theophylline in saliva, serum and ultrafiltrate for 10 healthy volunteers after administration of a single dose of a theophylline ethylenediamine solution are shown in figure 4. The mean theophylline concentration in saliva was 20.2% higher than that in ultrafiltrate. This difference was statistically significant ($p < 0.05$). The averaged concentration-time curves of theophylline in saliva, serum and ultrafiltrate for 10 healthy volunteers after administration of a single dose of choline theophyllinate tablets are shown in figure 5.

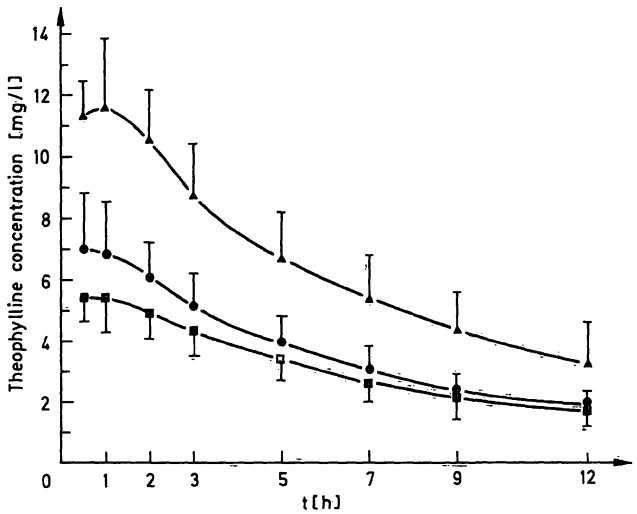

Fig. 4. Averaged concentration-time curves of theophylline in serum (▲—▲), saliva (●—●) and ultrafiltrate (■—■) after administration of a single dose of a theophylline ethylenediamine solution (5.6 mg/kg anhydrous theophylline) in healthy volunteers (n = 10). Vertical bars represent standard deviation.

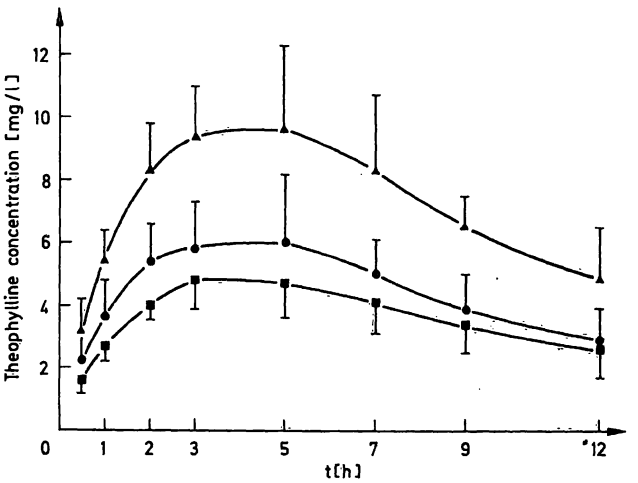

Fig. 5. Averaged concentration-time curves of theophylline in serum (▲—▲), saliva (●—●) and ultrafiltrate (■—■) after administration of a single dose of choline theophyllinate tablets (6.0 ± 0.6 mg/kg anhydrous theophylline) in healthy volunteers (n = 10). Vertical bars represent standard deviation.

The mean theophylline concentration in saliva was significantly 29.0% higher than that in ultrafiltrate ($p < 0.05$). Figure 6 shows the averaged concentration-time curves of theophylline in saliva, serum and ultrafiltrate for 8 healthy volunteers who received choline theophyllinate tablets for 3 days. The mean value of the theophylline concentrations in saliva was 19.2% higher than that in ultrafiltrate ($p < 0.05$).

Between 0.5 and 12 h after the administration of a single dose of the theophylline ethylenediamine solution or choline theophyllinate tablets, the saliva/serum ratios of the 10 volunteers decreased significantly from an average 0.63 to 0.55 and 0.73 to 0.60, respectively (tab. 3). The corresponding saliva/serum water concentration ratios of these subjects showed a statistically significant decrease from an average 1.29 to 1.05 and 1.39 to 1.11, respectively (tab. 3). However, at steady state the theophylline saliva/serum concentration ratios of theophylline determined between 0 to 12 h after administration of choline theophyllinate tablets did not show a significant difference ($p > 0.05$) (tab. 4). The saliva/serum water concentration ratio of theophylline increased significantly from an average 1.09 to 1.31 ($p < 0.05$).

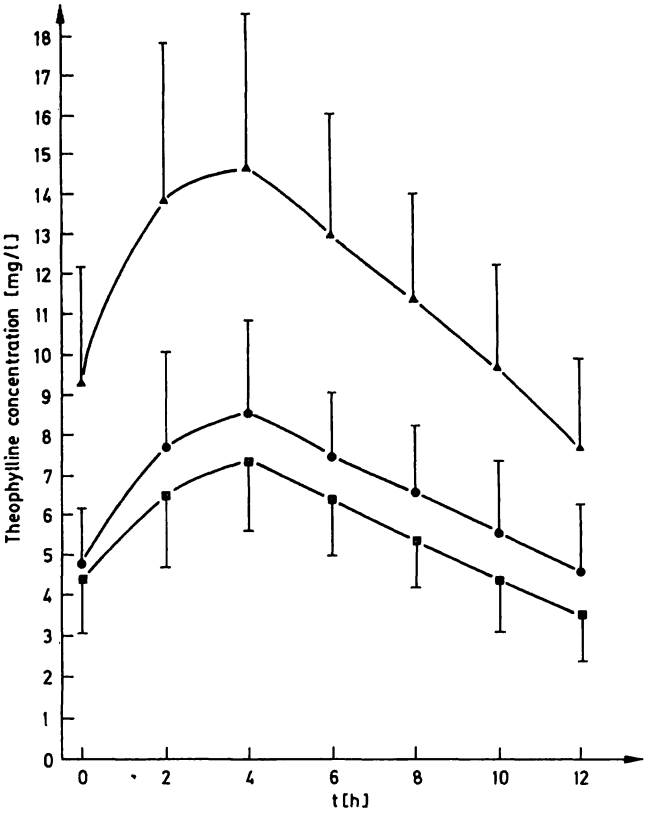

Fig. 6. Averaged concentration-time curves of theophylline in serum (▲—▲), saliva (●—●) and ultrafiltrate (■—■) at steady state after the administration of choline theophyllinate tablets at 8.00 a. m. and 8.00 p. m. for three days (5.7 ± 0.7 mg/kg anhydrous theophylline) in healthy volunteers (n = 8). Vertical bars represent standard deviation.
[theophylline bound to serum proteins: $\bar{x}$ (s) = 52.2 (4.3)%].

Tab. 3. The mean saliva/serum and saliva/serum water concentration ratio of theophylline with standard deviation in healthy volunteers (n = 10) 0.5 to 12 h after administration of a single dose of theophylline ethylenediamine solution (A) and choline theophyllinate tablets (B).

		0,5	1	2	3	5	7	9	12	h
A	a)	0.63 (0.14)	0.59 (0.08)	0.58 (0.09)	0.58 (0.09)	0.57 (0.08)	0.55 (0.09)	0.55 (0.07)	0.55 (0.07)	
	b)	1.29 (0.14)	1.27 (0.18)	1.26 (0.12)	1.24 (0.14)	1.18 (0.10)	1.18 (0.09)	1.13 (0.09)	1.05 (0.09)	
B	a)	0.73 (0.12)	0.68 (0.15)	0.65 (0.11)	0.63 (0.08)	0.60 (0.07)	0.60 (0.07)	0.60 (0.05)	0.60 (0.05)	
	b)	1.39 (0.09)	1.36 (0.18)	1.36 (0.17)	1.28 (0.09)	1.34 (0.16)	1.24 (0.16)	1.22 (0.09)	1.11 (0.14)	

a) saliva/serum concentration ratio of theophylline
b) saliva/serum water concentration ratio of theophylline

Tab. 4. The mean saliva/serum and saliva/serum water concentration ratio of theophylline with standard deviation in healthy volunteers (n = 8) 0 to 12 h after administration of choline theophyllinate tablets for 3 days.

	0	2	4	6	8	10	12	h
a)	0.50 (0.07)	0.50 (0.06)	0.51 (0.08)	0.52 (0.07)	0.51 (0.07)	0.54 (0.09)	0.53 (0.08)	
b)	1.09 (0.15)	1.18 (0.12)	1.16 (0.13)	1.17 (0.11)	1.20 (0.13)	1.24 (0.15)	1.31 (0.14)	

a) saliva/serum concentration ratio of theophylline
b) saliva/serum water concentration ratio of theophylline

In figure 7 serum theophylline concentrations obtained in samples from 30 outpatients are compared with the corresponding salivary theophylline concentrations. A high correlation was found (r = 0.97). The mean saliva/serum concentration ratio of theophylline was 0.68 ± 0.08 (range: 0.50–0.85). The serum theophylline concentrations of the 30 outpatients ranged from 1.6 to 17.3 mg/l, and the salivary concentrations from 0.7 to 9.5 mg/l. The serum water theophylline concentrations obtained in samples from these outpatients correlated well with the corresponding salivary theophylline concentrations as shown in figure 8 (r = 0.98). The mean saliva/serum water concentration ratio of theophylline was 1.34 ± 0.11 (range: 1.17–1.60).

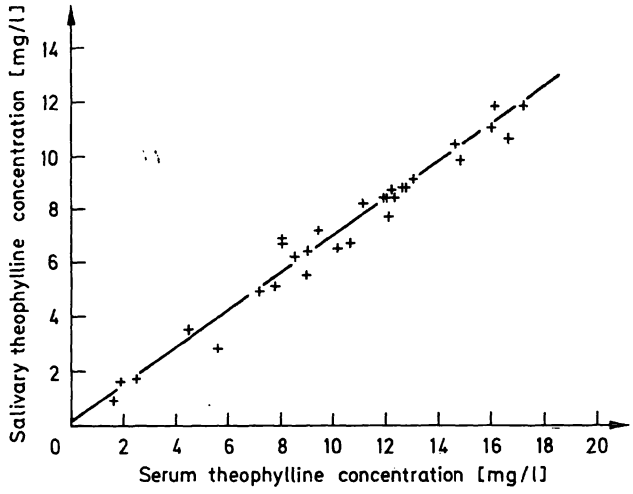

Fig. 7. Comparison of salivary theophylline concentrations with the corresponding serum theophylline concentrations in samples from outpatients (n = 30) treated with the drug.
[y = 0.68x + 0.01 mg/l; r = 0.97; $\bar{x}$ (s) = 10.5 (4.3) mg/l; $\bar{y}$ (s) = 7.1 (2.9) mg/l].

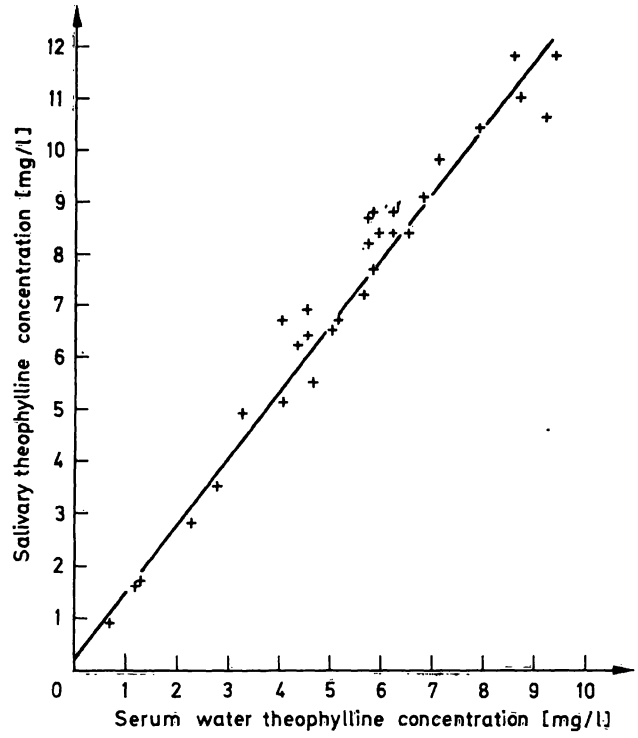

Fig. 8. Comparison of salivary theophylline concentrations with the corresponding serum water theophylline concentrations in samples from outpatients (n = 30) treated with the drug.
[y = 1.28x + 0.28 mg/l; r = 0.98; $\bar{x}$ (s) = 5.4 (2.3) mg/l; $\bar{y}$ (s) = 7.1 (2.9) mg/l].

Table 5 shows a comparison of predicted serum theophylline levels with the observed serum theophylline concentrations. The prediction is based on a mean saliva/serum concentration ratio of 0.68 obtained in 30 outpatients. A mean absolute prediction error of 7.6% (range: 0.0–26.8%) was found.

Tab. 5. Comparison between predicted (Css pred) and measured (Css m) steady state serum theophylline concentrations. In each patient serum theophylline concentrations were predicted from the corresponding salivary levels using a mean group saliva/serum concentration ratio of 0.68.

Patient	Css pred (mg/l)	Css m (mg/l)	% Dev.[1])	Patient	Css pred (mg/l)	Css m (mg/l)	% Dev.[1])
1	17.3	16.2	+ 6.8	16	14.4	14.9	− 3.4
2	7.5	7.8	− 3.9	17	11.3	12.2	− 7.4
3	15.3	14.7	+ 4.1	18	2.5	2.5	0.0
4	5.1	4.5	+13.3	19	12.3	12.0	+ 2.5
5	9.8	10.7	− 8.4	20	2.3	1.9	+21.1
6	12.9	12.7	+ 1.6	21	12.8	12.3	+ 4.1
7	10.1	8.1	+24.7	22	8.1	9.0	−10.0
8	9.1	8.6	+ 5.8	23	7.2	7.2	0.0
9	9.8	8.1	+21.0	24	1.3	1.6	−18.8
10	12.0	11.2	+ 7.1	25	12.3	12.1	+ 1.7
11	12.3	12.4	− 0.8	26	4.1	5.6	−26.8
12	9.4	9.1	+ 3.3	27	16.2	16.1	+ 0.6
13	16.2	17.3	− 6.4	28	9.6	10.2	− 5.9
14	15.6	16.7	− 6.6	29	10.6	11.2	− 5.4
15	12.9	12.8	+ 0.8	30	13.4	14.0	− 4.3
				$\bar{x}$ (s)	10.5 ± 4.3	10.5 ± 4.3	7.6[2])

[1]) % Dev. = $\frac{\text{Css pred} - \text{Css m}}{\text{Css m}} \cdot 100$ [2]) mean absolute % deviation

Discussion

The results of our study indicate that FPIA is very well suited for rapid and reliable theophylline determinations in saliva and serum water. Nineteen different samples from patients can be analysed by this method in about 10 minutes. Regarding the separation of unbound theophylline, the results obtained with the ultrafiltration technique agreed well with those using ultracentrifugation.

In order to obtain meaningful data on the unbound fraction of theophylline it is necessary to carefully control the pH value of the serum samples. In agreement with various other authors (13–15) we observed a distinct dependence of serum protein binding of theophylline upon serum pH (fig. 3). At a serum pH of 7.4 and a temperature of 25 °C we found a mean serum protein binding of about 50%. Somewhat higher values for the protein binding of theophylline have been observed in previous studies, in which the pH of samples was apparently not controlled (16). Since the pH increases in stored serum samples due to CO_2 release, falsely elevated values for the binding of theophylline to serum protein are obtained, if the pH of the sample is not adjusted to 7.4 (13).

In our study high overall correlation coefficients were found between salivary and serum theophylline levels ($r = 0.90$) and between salivary and unbound serum theophylline concentrations ($r = 0.91$) in healthy volunteers after administration of single theophylline doses and at steady state. During the first few hours after intravenous or oral administration of single theophylline doses, saliva/serum ratios were significantly higher and more variable than at the end of the experimental period of 12 hours (tab. 3). This phenomenon has also been described by *Koysooko* et al. (7) and *Uden* et al. (17). The possibility cannot be excluded that this finding is due to an active secretion of theophylline into saliva during the initial period (18). At steady state, however, no significant changes of the saliva/serum concentration ratio were observed during a 12 hour period between doses (tab. 4). So far, no explanation is available for the significant increase of the saliva/serum water concentration ratio at steady state (tab. 4). In samples from healthy volunteers and outpatients the theophylline concentration in saliva was, on average, about 20–30% higher than the unbound theophylline concentration in serum water. This finding is consistent with those of other authors (19). The concentration of theophylline in saliva is reportedly neither influenced by salivary pH nor flow rates (4, 5, 18, 20, 21). Moreover, only very little theophylline seems to be bound to salivary proteins (22).

A high correlation was found between salivary and serum as well as salivary and serum water theophylline concentrations in 30 outpatients treated with this drug (figs. 7, 8). The saliva/serum concentration ratio of theophylline determined in this study is consistent with those reported by various other authors (2, 3, 4, 6, 23). During a test period of 12 hours no relevant intrasubject change of this ratio was detected (tabs. 3, 4). Data from the literature (2, 7) also indicate that in general there is only slight intrasubject variability of this ratio from week to week. However, in agreement with various other investigators (3, 6, 17, 23), some intersubject variation of the saliva/serum concentration ratio of theophylline was found (range: 0.50–0.85). Using the average saliva/serum concentration ratio of theophylline obtained with samples from these patients, predicted serum theophylline levels were compared with the observed measured values (tab. 5). A mean absolute deviation of 7.6% between predicted and measured serum theophylline concentrations was found. The observed deviations ranged from 0–27% with only 4 patients out of 30 showing a prediction error between 20 and 27%. However, serum theophylline levels predicted from salivary theophylline concentrations should be interpreted carefully, if the individual saliva/serum ratio of a patient is not known. The wide interlaboratory variation in saliva/serum concentration ratios is at least partly due to different methods of saliva collection. Standardised stimulation of salivation as described in this study might also help to reduce intralaboratory variation of this ratio.

From the results of our study we conclude that the theophylline concentration in stimulated saliva is a clinically valuable parameter for monitoring theophylline therapy. When interpreting salivary theophylline levels, however, the intersubject variability of the saliva/serum concentration ratio of this substance should be taken into account. Salivary theophylline concentrations are useful for the assessment of compliance and the identification of patients who receive inadequate or excessive theophylline doses. This non-invasive method is of particular interest for the large number of outpatients with nocturnal or unstable asthma, as it allows theophylline determinations at any time during the dosing interval. Monitoring of salivary theophylline concentrations could identify the role of theophylline in the overall drug regimen especially before embarking on steroid treatment. The individual dosage optimisation and the verification of suspected overdosage, however, should always be based on theophylline concentrations determined in serum.

References

1. Hendeles, L., Weinberger, M. & Johnson, G. (1978) J. Pediatrics *96*, 494–499.
2. Galant, S. P., Gillmann, S. A., Cummins, L. H., Kozek, P. P. & Orcutt, J. J. (1977) Am. J. Dis. Child. *131*, 970–972.
3. Kelly, H. W., Hadley, W. M., Murphy, S. A. & Skipper, B. G. (1981) Am. J. Dis. Child. *135*, 137–139.
4. Levy, G., Ellis, E. F. & Koysooko, R. (1974) Pediatrics *53*, 873–876.
5. Boobis, S., Trembath, P. W., Chambers, R. E., Edmunds, A. T. & Carswell, F. (1979) Ther. Drug Monitoring *1*, 485–493.
6. Hendeles, L., Burkey, S., Bighly, L. & Richardson, R. (1977) J. Allergy Clin. Immunol. *60*, 335–338.
7. Koysooko, R., Ellis, E. F. & Levy, G. (1974) Clin. Pharmacol. Ther. *15*, 454–460.
8. Neuenkirchen, H., Wilkens, J. H., Oellerich, M. & Sybrecht, G. W. (1985) Eur. J. Respir. Dis. *66*, 196–204.
9. Oellerich, M. & Mueller-Vahl, H. (1984) Clin. Pharmacokinet. *9*, 61–70.
10. Schumann, G., Oellerich, M. & Sybrecht, G. W. J. Clin. Chem. Clin. Biochem. In prep.
11. Schumann, G., Isberner, I. & Oellerich, M. (1984) Fresenius Z Anal Chem. *317*, 677.
12. Feldmann, U., Schneider, B., Klinkers, H. & Haeckel, R. (1981) J. Clin. Chem. Clin. Biochem. *19*, 121–137.
13. Brors, O., Sager, G., Sandness, D. & Jacobson, S. (1983) Br. J. Clin. Pharmacol. *15*, 393–397.
14. Shaw, L. M., Fields, L. & Maycock, R. (1982) Clin. Pharmacol. Ther. *32*, 490–496.
15. Vallner, J. J., Speir, W. A., Kolbeck, R. C., Harrison, G. N. & Bransome, E. D. (1979) Am. Rev. Respir. Dis. *120*, 83–86.
16. Simons, K. J., Simons, F. E. R., Briggs, C. J. & Lo, L. (1979) J. Pharmaceut. Sci. *68*, 252–253.
17. Uden, D. L., Miller, K. W., Strand, L. M. Johnson, P. B. & Zaske, D. E. (1981) Ther. Drug Monit. *3*, 143–150.
18. Danhof, M. & Breimer, D. D. (1978) Clin. Pharmacokinet. *3*, 39–57.
19. Knott, C., Batemann, M. & Reynolds, F. (1984) Br. J. Clin. Pharmacol. *17*, 9–14.
20. Jackson, A. H., Edwards, E., Cope, A. S., Stockley, R. A. & Purkiss, R. (1983) Br. J. Clin. Pharmacol. *15*, 407–410.
21. Culig, J., Johnston, A. & Turner, P. (1982) Br. J. Clin. Pharmacol. *13*, 243–245.
22. Khanna, N. N., Bada, H. S. & Somani, S. M. (1980) J. Pediatrics *96*, 494–499.
23. Lena, S. M., Hutchins, P., Wood, C. B. S. & Turner, P. (1980) Postgrad. Med. J. *56*, 85–87.

Prof. Dr. M. Oellerich
Institut für Klinische Chemie
Medizinische Hochschule Hannover
Konstanty-Gutschow-Straße 8
D-3000 Hannover 61
Federal Republic of Germany